L Number	Hits	Search Text	DB	Time stamp
1	451	endothelial and (nitric adj1 oxide) and (antibody near10 phosphoryla$4)	USPAT; US-PGPUB; EPO; DERWENT	2004/04/14 12:19
2	4	(nitric adj1 oxide) same (antibody near10 phosphoryla$4)	USPAT; US-PGPUB; EPO; DERWENT	2004/04/14 12:18
3	1	(endothelial and (nitric adj1 oxide) and (antibody near10 phosphoryla$4)) and ((nitric adj1 oxide) same (antibody near10 phosphoryla$4))	USPAT; US-PGPUB; EPO; DERWENT	2004/04/14 12:18
4	34	endothelial same (antibody near10 phosphoryla$4)	USPAT; US-PGPUB; EPO; DERWENT	2004/04/14 12:19
5	14	(endothelial and (nitric adj1 oxide) and (antibody near10 phosphoryla$4)) and (endothelial same (antibody near10 phosphoryla$4))	USPAT; US-PGPUB; EPO; DERWENT	2004/04/14 12:19

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COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

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FILE 'BIOTECHNO' ENTERED AT 12:03:12 ON 14 APR 2004
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=> Zou and nitrix oxide and antibody
L1            0 FILE AGRICOLA
L2            0 FILE BIOTECHNO
L3            0 FILE CONFSCI
L4            0 FILE HEALSAFE
L5            0 FILE IMSDRUGCONF
L6            0 FILE LIFESCI
L7            0 FILE MEDICONF
L8            0 FILE PASCAL

TOTAL FOR ALL FILES
L9            0 ZOU AND NITRIX OXIDE AND ANTIBODY

=> nitrix oxide and antibody and (phosphorylated or phosphorylation)
L10           0 FILE AGRICOLA
L11           0 FILE BIOTECHNO
L12           0 FILE CONFSCI
L13           0 FILE HEALSAFE
L14           0 FILE IMSDRUGCONF
L15           0 FILE LIFESCI
L16           0 FILE MEDICONF
L17           0 FILE PASCAL

TOTAL FOR ALL FILES
L18           0 NITRIX OXIDE AND ANTIBODY AND (PHOSPHORYLATED OR PHOSPHORYLATION
                )

=> nitric oxide and antibody and (phosphorylated or phosphorylation)
L19           1 FILE AGRICOLA
L20          60 FILE BIOTECHNO
L21           0 FILE CONFSCI
L22           0 FILE HEALSAFE
```

```
L23           0 FILE IMSDRUGCONF
L24          26 FILE LIFESCI
L25           0 FILE MEDICONF
L26          39 FILE PASCAL

TOTAL FOR ALL FILES
L27         126 NITRIC OXIDE AND ANTIBODY AND (PHOSPHORYLATED OR PHOSPHORYLATION
                )

=>  antibody(8A)(phosphorylated or phosphorylation)
L28          62 FILE AGRICOLA
L29        1567 FILE BIOTECHNO
L30          22 FILE CONFSCI
L31           0 FILE HEALSAFE
L32           0 FILE IMSDRUGCONF
L33        1145 FILE LIFESCI
L34           0 FILE MEDICONF
L35         924 FILE PASCAL

TOTAL FOR ALL FILES
L36        3720 ANTIBODY(8A)(PHOSPHORYLATED OR PHOSPHORYLATION)

=> l27 and l36
L37           1 FILE AGRICOLA
L38          19 FILE BIOTECHNO
L39           0 FILE CONFSCI
L40           0 FILE HEALSAFE
L41           0 FILE IMSDRUGCONF
L42          10 FILE LIFESCI
L43           0 FILE MEDICONF
L44          15 FILE PASCAL

TOTAL FOR ALL FILES
L45          45 L27 AND L36

=> l45 and endothelial
L46           0 FILE AGRICOLA
L47           7 FILE BIOTECHNO
L48           0 FILE CONFSCI
L49           0 FILE HEALSAFE
L50           0 FILE IMSDRUGCONF
L51           1 FILE LIFESCI
L52           0 FILE MEDICONF
L53           3 FILE PASCAL

TOTAL FOR ALL FILES
L54          11 L45 AND ENDOTHELIAL

=> dup rem
ENTER L# LIST OR (END):l54
DUPLICATE IS NOT AVAILABLE IN 'IMSDRUGCONF, MEDICONF'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L54
L55           9 DUP REM L54 (2 DUPLICATES REMOVED)

=> d l9 ibib abs total
L9 HAS NO ANSWERS
'TOTAL ' IS NOT A VALID SEARCH STATUS KEYWORD
Search status keywords:
NONE ---- Display only the number of postings.
STATUS -- Display statistics of the search.
ENTER SEARCH STATUS OPTION (NONE), STATUS, OR ?:none
L1            0 SEA FILE=AGRICOLA ABB=ON  PLU=ON  ZOU AND NITRIX OXIDE AND
                ANTIBODY
```

L2 0 SEA FILE=BIOTECHNO ABB=ON PLU=ON ZOU AND NITRIX OXIDE AND ANTIBODY
L3 0 SEA FILE=CONFSCI ABB=ON PLU=ON ZOU AND NITRIX OXIDE AND ANTIBODY
L4 0 SEA FILE=HEALSAFE ABB=ON PLU=ON ZOU AND NITRIX OXIDE AND ANTIBODY
L5 0 SEA FILE=IMSDRUGCONF ABB=ON PLU=ON ZOU AND NITRIX OXIDE AND ANTIBODY
L6 0 SEA FILE=LIFESCI ABB=ON PLU=ON ZOU AND NITRIX OXIDE AND ANTIBODY
L7 0 SEA FILE=MEDICONF ABB=ON PLU=ON ZOU AND NITRIX OXIDE AND ANTIBODY
L8 0 SEA FILE=PASCAL ABB=ON PLU=ON ZOU AND NITRIX OXIDE AND ANTIBODY
L9 0 SEA ZOU AND NITRIX OXIDE AND ANTIBODY

=> d l54 ibib abs total

L54 ANSWER 1 OF 11 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 2003:36898649 BIOTECHNO
TITLE: Regulation of **endothelial nitric oxide** synthase by protein kinase C
AUTHOR: Matsubara M.; Hayashi N.; Jing T.; Titani K.
CORPORATE SOURCE: M. Matsubara, R and D Laboratories, Nippon Organon K.K., 1-5-90 Tomobuchi-cho, Osaka, 534-0016, Japan. E-mail: Mamoru.Matsubara@organon.jp
SOURCE: Journal of Biochemistry, (01 JUN 2003), 133/6 (773-781), 53 reference(s) CODEN: JOBIAO ISSN: 0021-924X
DOCUMENT TYPE: Journal; Article
COUNTRY: Japan
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2003:36898649 BIOTECHNO
AB **Endothelial nitric oxide** synthase (eNOS) is a key enzyme in **nitric oxide**-mediated signal transduction in mammalian cells. Its catalytic activity is regulated both by regulatory proteins, such as calmodulin and caveolin, and by a variety of post-translational modifications including **phosphorylation** and acylation. We have previously shown that the calmodulin-binding domain peptide is a good substrate for protein kinase C [Matsubara, M., Titani, K., and Taniguchi, H. (1996) Biochemistry 35, 14651-14658]. Here we report that bovine eNOS protein is **phosphorylated** at Thr497 in the calmodulin-binding domain by PKC both in vitro and in vivo, and that the **phosphorylation** negatively regulates eNOS activity. A specific **antibody** that recognizes only the **phosphorylated** form of the enzyme was raised against a synthetic phosphopeptide corresponding to the **phosphorylated** domain. The **antibody** recognized eNOS immunoprecipitated with anti-eNOS **antibody** from the soluble fraction of bovine aortic **endothelial** cells, and the immunoreactivity increased markedly when the cells were treated with phorbol 12-myristate 13-acetate. PKC **phosphorylated** eNOS specifically at Thr497 with a concomitant decrease in the NOS activity. Furthermore, the **phosphorylated** eNOS showed reduced affinity to calmodulin. Therefore, PKC regulates eNOS activity by changing the binding of calmodulin, an eNOS activator, to the enzyme.

L54 ANSWER 2 OF 11 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 2002:35190934 BIOTECHNO
TITLE: Subcellular targeting and agonist-induced site-specific **phosphorylation** of **endothelial nitric-oxide**

synthase
AUTHOR: Gonzalez E.; Kou R.; Lin A.J.; Golan D.E.; Michel T.
CORPORATE SOURCE: T. Michel, Cardiovascular Division, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115, United States.
E-mail: michel@calvin.bwh.harvard.edu
SOURCE: Journal of Biological Chemistry, (18 OCT 2002), 277/42 (39554-39560), 39 reference(s)
CODEN: JBCHA3 ISSN: 0021-9258
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2002:35190934 BIOTECHNO
AB The **endothelial** isoform of **nitric-oxide** synthase (eNOS) undergoes a complex pattern of covalent modifications, including acylation with the fatty acids myristate and palmitate as well as **phosphorylation** on multiple sites. eNOS acylation is a key determinant for the reversible subcellular targeting of the enzyme to plasmalemmal caveolae. We transfected a series of hemagglutinin epitope-tagged eNOS mutant cDNAs deficient in palmitoylation (palm.sup.-) and/or myristoylation (myr.sup.-) into bovine aortic **endothelial** cells; after treatment with the eNOS agonists sphingosine 1-phosphate or vascular **endothelial** growth factor, the recombinant eNOS was immunoprecipitated using an **antibody** directed against the epitope tag, and patterns of eNOS **phosphorylation** were analyzed in immunoblots probed with **phosphorylation** state-specific eNOS **antibodies**. The wild-type eNOS underwent agonist-induced **phosphorylation** at serine 1179 (a putative site for **phosphorylation** by kinase Akt), but **phosphorylation** of the myr.sup.- eNOS at this residue was nearly abrogated; the palm.sup.- eNOS exhibited an intermediate phenotype. The addition of the CD8 transmembrane domain to the amino terminus of eNOS acylation-deficient mutants rescued the wild-type phenotype of robust agonist-induced serine 1179 **phosphorylation**. Thus, membrane targeting, but not necessarily acylation, is the critical determinant for agonist-promoted eNOS **phosphorylation** at serine 1179. In striking contrast to serine 1179, **phosphorylation** of eNOS at serine 116 was enhanced in the myr.sup.- eNOS mutant and was markedly attenuated in the CD8-eNOS membrane-targeted fusion protein. We conclude that eNOS targeting differentially affects eNOS **phosphorylation** at distinct sites in the protein and suggest that the inter-relationships of eNOS acylation and **phosphorylation** may modulate eNOS localization and activity and thereby influence NO signaling pathways in the vessel wall.

L54 ANSWER 3 OF 11 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 2002:34112805 BIOTECHNO
TITLE: **Phosphorylation** of blood vessel vasodilator-stimulated phosphoprotein at Serine 239 as a functional biochemical marker of **endothelial** **nitric oxide**/cyclic GMP signaling
AUTHOR: Ibarra-Alvarado C.; Galle J.; Melichar V.O.; Mameghani A.; Schmidt H.H.H.W.
CORPORATE SOURCE: Dr. C. Ibarra-Alvarado, Rudolf-Buchheim-Inst. of Pharmacol., Justus-Liebig-University, Frankfurter Strasse 107, 35392 Giessen, Germany.
E-mail: cesar.ibarra@pharma.med.uni-giessen.de
SOURCE: Molecular Pharmacology, (2002), 61/2 (312-319), 40 reference(s)
CODEN: MOPMA3 ISSN: 0026-895X
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English

AN 2002:34112805 BIOTECHNO
AB The endothelium-derived relaxing factors **nitric oxide** (NO) and prostacyclin (PGI.sub.2) are important antithrombotic, relaxant, and antiproliferative agents of the blood vessel wall that exert their intracellular effects primarily via cGMP- and cAMP-dependent protein kinases (cGK, cAK). However, no biochemical marker for their activity in the intact blood vessel is available except for transient increases in the concentration of cGMP and cAMP. Using Western blot analysis and specific **antibodies**, we show here that **phosphorylation** of the vasodilator-stimulated phosphoprotein (VASP) at Ser239 (P.sub.S.sub.e.sub.r.sub.2.sub.3.sub.9-VASP) in rabbit aorta was detectable only in segments with an intact endothelium, although at least one third of VASP is contained in the remaining vascular wall. In endothelium-denuded aorta, VASP **phosphorylation** was increased by the NO donor sodium nitroprusside (SNP). Levels of P.sub.S.sub.e.sub.r.sub.2.sub.3.sub.9-VASP, in the presence of endothelium and either SNP or 8-bromo-cAMP, were maximal. VASP **phosphorylation** elicited by 8-bromo-cAMP was inhibited significantly by the cGK inhibitor Rp-8-Br-PET-cGMPS. Stimulated P.sub.S.sub.e.sub.r.sub.2.sub.3.sub.9-VASP formation was fully reversible, reaching basal levels after 10 min of repeated washouts. Consistent with the important role that the NO/cGMP pathway plays in the formation of P.sub.S.sub.e.sub.r.sub.2.sub.3.sub.9-VASP in rabbit aorta, inhibition of NO synthase by N.sup.&.sup.o.sup.m.sup.e.sup.g.sup.a.sup.;-nitro-L-arginine methyl ester (L-NAME; 1 mM) or of soluble guanylyl cyclase by 1H-[1,2,4]oxadiazolo[3,4-a]quinoxalin-1-one (ODQ; 50 μM) almost completely abolished P.sub.S.sub.e.sub.r.sub.2.sub.3.sub.9-VASP formation in endothelium intact blood vessels. These data suggest that vascular P.sub.S.sub.e.sub.r.sub.2.sub.3.sub.9-VASP is primarily regulated by the NO/cGMP pathway and may thus serve as a biochemical marker for the activity state of this essential pathway in **endothelial** function.

L54 ANSWER 4 OF 11 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 2002:34106380 BIOTECHNO
TITLE: Activation of eNOS in rat portal hypertensive gastric mucosa is mediated by TNF-α via the PI 3-kinase-Akt signaling pathway
AUTHOR: Kawanaka H.; Jones M.K.; Szabo I.L.; Baatar D.; Pai R.; Tsugawa K.; Sugimachi K.; Sarfeh I.J.; Tarnawski A.S.
CORPORATE SOURCE: Dr. A.S. Tarnawski, Gastroenterology Section (111G), DVA Medical Center, 5901 E. Seventh St, Long Beach, CA 90822, United States.
E-mail: atarnawski@yahoo.com
SOURCE: Hepatology, (2002), 35/2 (393-402), 50 reference(s)
CODEN: HPTLD0 ISSN: 0270-9139
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2002:34106380 BIOTECHNO
AB Activation of **endothelial nitric oxide** synthase (eNOS) in portal hypertensive (PHT) gastric mucosa leads to hyperdynamic circulation and increased susceptibility to injury. However, the signaling mechanisms for eNOS activation in PHT gastric mucosa and the role of TNF-α in this signaling remain unknown. In PHT gastric mucosa we studied (1) eNOS **phosphorylation** (at serine 1177) required for its activation; (2) association of the phosphatidylinositol 3-kinase (PI 3-kinase), and its downstream effector Akt, with eNOS; and, (3) whether TNF-α neutralization affects eNOS **phosphorylation** and PI 3-kinase-Akt activation. To determine human relevance, we used human microvascular **endothelial** cells to examine directly whether TNF-α stimulates eNOS

phosphorylation via PI 3-kinase. PHT gastric mucosa has significantly increased (1) eNOS **phosphorylation** at serine 1177 by 90% (P < .01); (2) membrane translocation (P < .05) and **phosphorylation** (P < .05) of p85 (regulatory subunit of PI 3-kinase) by 61% and 85%, respectively; (3) **phosphorylation** (P < .01) and activity (P < .01) of Akt by 40% and 52%, respectively; and (4) binding of Akt to eNOS by as much as 410% (P < .001). Neutralizing anti-TNF-α **antibody** significantly reduced p85 **phosphorylation**, **phosphorylation** and activity of Akt, and eNOS **phosphorylation** in PHT gastric mucosa to normal levels. Furthermore, TNF-α stimulated eNOS **phosphorylation** in human microvascular **endothelial** cells. In conclusion, these findings show that in PHT gastric mucosa, TNF-α stimulates eNOS **phosphorylation** at serine 1177 (required for its activation) via the PI 3-kinase-Akt signal transduction pathway.

L54 ANSWER 5 OF 11 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 2000:34017502 BIOTECHNO
TITLE: Role of heat shock protein 90 in bradykinin-stimulated **endothelial nitric oxide** release
AUTHOR: Harris M.B.; Ju H.; Venema V.J.; Blackstone M.; Venema R.C.
CORPORATE SOURCE: R.C. Venema, Vascular Biology Center, Medical College of Georgia, CB 3207, 1459 Laney Walker Boulevard, Augusta, GA 30912-2500, United States.
E-mail: rvenema@mail.mcg.edu
SOURCE: General Pharmacology: The Vascular System, (2000), 35/3 (165-170), 19 reference(s)
CODEN: GEPHDP ISSN: 0306-3623
PUBLISHER ITEM IDENT.: S0306362301001045
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2000:34017502 BIOTECHNO
AB Previously we described ENAP-1, a 90-kDa protein that is tyrosine-**phosphorylated** in **endothelial** cells in response to bradykinin (BK) stimulation and is associated with **endothelial nitric oxide** synthase (eNOS). Subsequently, other investigators demonstrated that eNOS interacts with heat shock protein 90 (Hsp90) following stimulation of **endothelial** cells with vascular **endothelial** growth factor (VEGF), histamine, or fluid shear stress. Therefore, we tested the hypotheses that ENAP-1 and Hsp90 are the same protein and that BK activation of eNOS is dependent on Hsp90. Immunoblotting of immunoprecipitated Hsp90 with anti-phosphotyrosine **antibody** shows that Hsp90 is tyrosine-**phosphorylated** in response to BK stimulation of bovine aortic **endothelial** cells (BAECs). Coimmunoprecipitation of Hsp90 with anti-eNOS **antibody** reveals a Hsp90-eNOS complex in **endothelial** cells under basal conditions that is increased following BK stimulation. Taken together with the tyrosine **phosphorylation** data, these data suggest that ENAP-1 is Hsp90. BK-stimulated **nitric oxide** (NO) release is completely blocked by pretreatment with geldanamycin, a specific inhibitor of Hsp90, illustrating the importance of the Hsp90-eNOS interaction. In vitro binding assays with Hsp90-glutathione-S-transferase fusion proteins show direct binding of eNOS with the middle domain (residues 259-615) of Hsp90.

L54 ANSWER 6 OF 11 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1996:26348536 BIOTECHNO
TITLE: Bradykinin-stimulated protein tyrosine **phosphorylation** promotes **endothelial**

nitric oxide synthase translocation to the cytoskeleton
AUTHOR: Venema V.J.; Marrero M.B.; Venema R.C.
CORPORATE SOURCE: Vascular Biology Center, Medical College of Georgia,Augusta, GA 30912-2500, United States.
SOURCE: Biochemical and Biophysical Research Communications, (1996), 226/3 (703-710)
CODEN: BBRCA0 ISSN: 0006-291X
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1996:26348536 BIOTECHNO
AB Stimulation of bovine aortic **endothelial** cells (BAEC) with bradykinin produces cycles of tyrosine **phosphorylation** /dephosphorylation of a 90 kDa **endothelial nitric oxide** synthase (eNOS)-associated protein which we have termed ENAP-1 (for **endothelial nitric oxide** synthase-associated protein 1). ENAP-1 interacts specifically and tightly with eNOS in BAEC and is co-immunoprecipitated from cell lysates with anti-eNOS **antibodies**. In addition, anti-phosphotyrosine **antibodies** co-precipitate eNOS. Bradykinin-stimulated tyrosine **phosphorylation** of ENAP-1 is blocked by the tyrosine kinase inhibitor, tyrphostin. Dephosphorylation is blocked by the tyrosine phosphatase inhibitor, orthovanadate. Treatment of BAEC with bradykinin or the tyrosine phosphatase inhibitor, phenylarsine oxide promotes tyrosine **phosphorylation** of detergent-insoluble, cytoskeletal proteins accompanied by translocation of eNOS to the cytoskeletal subcellular compartment. Translocation is blocked by the tyrosine kinase inhibitor, geldanamycin and does not appear to alter enzyme catalytic activity. Tyrosine **phosphorylation**-dependent association of eNOS with the cytoskeleton may have a role in targeting NO production to specific subcellular locations.

L54 ANSWER 7 OF 11 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1993:23191493 BIOTECHNO
TITLE: **Phosphorylation** and subcellular translocation of **endothelial nitric oxide** synthase
AUTHOR: Michel T.; Li G.K.; Busconi L.
CORPORATE SOURCE: Thorn Building 1110A, Brigham and Women's Hospital, 75 Francis Street,Boston, MA 02115, United States.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1993), 90/13 (6252-6256)
CODEN: PNASA6 ISSN: 0027-8424
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1993:23191493 BIOTECHNO
AB In the vascular endothelium, diverse cell surface receptors are coupled to the Ca.sup.2.sup.+/calmodulin-dependent activation of **nitric oxide** (NO) synthase. We now report that, in intact cultured **endothelial** cells, several drugs and agonists are associated with increased serine **phosphorylation** of the **endothelial** NO synthase. We biosynthetically labeled bovine aortic **endothelial** cells with ¢.sup.3.sup.2P!orthophosphoric acid, exposed the cells to various drugs and hormones, and then immunoprecipitated the enzyme from cell extracts using a highly specific anti-peptide **antibody**. The marked **endothelial** NO synthase **phosphorylation** induced by bradykinin is maximal only after 5 min of agonist exposure and is stable for at least 20 min. Basal and agonist-induced **phosphorylation** of the NO synthase in **endothelial** cells is completely inhibited by the calmodulin

antagonist compound W-7. We prepared subcellular fractions of **endothelial** cells that had been biosynthetically labeled with ¢.sup.3.sup.5S!methionine or ¢.sup.3.sup.2P!orthophosphoric acid and immunoprecipitated the **endothelial** NO synthase from untreated (basal) and bradykinin-treated cells. In the basal state, ¢.sup.3.sup.5S!methionine-labeled **endothelial** NO synthase is associated primarily with the particulate cellular fraction, but the **phosphorylated** enzyme is primarily cytosolic. Following exposure to bradykinin, a substantial fraction of the ¢.sup.3.sup.5S!methionine- labeled NO synthase is now found in the cytosolic fraction, associated with a marked increase in the level of cytosolic enzyme **phosphorylation**. We propose that agonist-induced **phosphorylation** of NO synthase is associated with translocation of the enzyme from membrane to cytosol and may thereby regulate the biological effects of **endothelial** NO synthesis in situ.

L54 ANSWER 8 OF 11 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 2002:106720 LIFESCI
TITLE: Dephosphorylation of **Endothelial Nitric -oxide** Synthase by Vascular **Endothelial** Growth Factor: Implications for the Vascular Responses to Cyclosporin A
AUTHOR: Kou, R.; Greif, D.; Michel, T.
CORPORATE SOURCE: Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115; E-mail: michel@calvin.bwh.harvard.edu
SOURCE: Journal of Biological Chemistry [J. Biol. Chem.], (20020816) vol. 277, no. 33, pp. 29669-29673. ISSN: 0021-9258.
DOCUMENT TYPE: Journal
FILE SEGMENT: X
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The **endothelial** isoform of **nitric-oxide** synthase (eNOS) is a key determinant of vascular tone. eNOS, a Ca super(2+)/camodulin-dependent enzyme, is also regulated by a variety of agonist-activated protein kinases, but the role and regulation of the protein phosphatase pathways involved in eNOS dephosphorylation are much less well understood. Treatment of **endothelial** cells with vascular **endothelial** growth factor (VEGF), a potent eNOS agonist, leads to the activation of calcineurin, a Ca super(2+)/camodulin-dependent protein phosphatase. In these studies, we used a **phosphorylation** state-specific **antibody** to show that VEGF promotes dephosphorylation of eNOS at serine residue 116 in cultured **endothelial** cells. Cyclosporin A, an inhibitor of calcineurin, completely blocks VEGF-induced eNOS dephosphorylation; under identical conditions, cyclosporin A also inhibits VEGF-induced eNOS activation. VEGF-induced eNOS dephosphorylation shows an EC sub(50) of 2 ng/ml and is maximal 30 min after agonist addition. eNOS **phosphorylation** at serine 116 is completely blocked by the protein kinase C inhibitor calphostin but is blocked by neither wortmannin (an inhibitor of phosphatidylinositide 3-kinase) nor the MAP kinase pathway inhibitor U0126. A **phosphorylation**-deficient mutant of eNOS in which serine 116 is changed to an alanine residue (S116A) shows significantly enhanced enzyme activity compared with the wild-type enzyme. Taken together, these findings indicated that VEGF-induced eNOS dephosphorylation at serine 116 leads to enzyme activation. Cyclosporin A is widely used as an immunosuppressive drug for which hypertension is an important dose-limiting side effect. Our results suggest that cyclosporin A-induced hypertension may involve, at least in part, the attenuation of endothelium-derived NO production through a calcineurin-sensitive pathway regulating eNOS dephosphorylation.

L54 ANSWER 9 OF 11 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002-0398416 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 2002 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): Activation of eNOS in rat portal hypertensive gastric mucosa is mediated by TNF-α via the PI 3-kinase-Akt signaling pathway
AUTHOR: KAWANAKA Hirofumi; JONES Michael K.; SZABO Imre L.; BAATAR Dolgor; PAI Rama; TSUGAWA Kouji; SUGIMACHI Keizo; SARFEH I. James; TARNAWSKI Andrzej S.
CORPORATE SOURCE: Departments of Medicine and Surgery, Department of Veterans Affairs Medical Center, Long Beach, United States; University of California, Irvine, CA, United States; Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
SOURCE: Hepatology : (Baltimore, Md.), (2002), 35(2), 393-402, 50 refs.
ISSN: 0270-9139 CODEN: HPTLD9
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-19427, 354000108716230190

AN 2002-0398416 PASCAL
CP Copyright .COPYRGT. 2002 INIST-CNRS. All rights reserved.
AB Activation of **endothelial nitric oxide** synthase (eNOS) in portal hypertensive (PHT) gastric mucosa leads to hyperdynamic circulation and increased susceptibility to injury. However, the signaling mechanisms for eNOS activation in PHT gastric mucosa and the role of TNF-α in this signaling remain unknown. In PHT gastric mucosa we studied (1) eNOS **phosphorylation** (at serine 1177) required for its activation; (2) association of the phosphatidylinositol 3-kinase (PI 3-kinase), and its downstream effector Akt, with eNOS; and, (3) whether TNF-a neutralization affects eNOS **phosphorylation** and PI 3-kinase-Akt activation. To determine human relevance, we used human microvascular **endothelial** cells to examine directly whether TNF-a stimulates eNOS **phosphorylation** via PI 3-kinase. PHT gastric mucosa has significantly increased (1) eNOS **phosphorylation** at serine 1177 by 90% ($P < .01$); (2) membrane translocation ($P < .05$) and **phosphorylation** ($P < .05$) of p85 (regulatory subunit of PI 3-kinase) by 61% and 85%, respectively; (3) **phosphorylation** ($P < .01$) and activity ($P < .01$) of Akt by 40% and 52%, respectively; and (4) binding of Akt to eNOS by as much as 410% ($P < .001$). Neutralizing anti-TNF-a **antibody** significantly reduced p85 **phosphorylation**, **phosphorylation** and activity of Akt, and eNOS **phosphorylation** in PHT gastric mucosa to normal levels. Furthermore, TNF-a stimulated eNOS **phosphorylation** in human microvascular **endothelial** cells. In conclusion, these findings show that in PHT gastric mucosa, TNF-a stimulates eNOS **phosphorylation** at serine 1177 (required for its activation) via the PI 3-kinase-Akt signal transduction pathway.

L54 ANSWER 10 OF 11 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002-0134647 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 2002 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): Role of heat shock protein 90 in bradykinin-stimulated **endothelial nitric oxide** release
Vascular papers
AUTHOR: HARRIS M. Brennan; HONG JU; VENEMA Virginia J.;

BLACKSTONE Michele; VENEMA Richard C.
CORPORATE SOURCE: Vascular Biology Center, Medical College of Georgia, Augusta, GA 30912-2500, United States; Department of Pediatrics, Medical College of Georgia, Augusta, GA 30912-2500, United States; Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2500, United States
SOURCE: General pharmacology, (2000), 35(3), 165-170, 19 refs.
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AN 2002-0134647 PASCAL
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AB Previously we described ENAP-1, a 90-kDa protein that is tyrosine-**phosphorylated** in **endothelial** cells in response to bradykinin (BK) stimulation and is associated with **endothelial** **nitric oxide** synthase (eNOS). Subsequently, other investigators demonstrated that eNOS interacts with heat shock protein 90 (Hsp90) following stimulation of **endothelial** cells with vascular **endothelial** growth factor (VEGF), histamine, or fluid shear stress. Therefore, we tested the hypotheses that ENAP-1 and Hsp90 are the same protein and that BK activation of eNOS is dependent on Hsp90. Immunoblotting of immunoprecipitated Hsp90 with anti-phosphotyrosine **antibody** shows that Hsp90 is tyrosine-**phosphorylated** in response to BK stimulation of bovine aortic **endothelial** cells (BAECs). Coimmunoprecipitation of Hsp90 with anti-eNOS **antibody** reveals a Hsp90-eNOS complex in **endothelial** cells under basal conditions that is increased following BK stimulation. Taken together with the tyrosine **phosphorylation** data, these data suggest that ENAP- 1 is Hsp90. BK-stimulated **nitric oxide** (NO) release is completely blocked by pretreatment with geldanamycin, a specific inhibitor of Hsp90, illustrating the importance of the Hsp90-eNOS interaction. In vitro binding assays with Hsp90-glutathione-3-transferase fusion proteins show direct binding of eNOS with the middle domain (residues 259-615) of Hsp90.

L54 ANSWER 11 OF 11 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2000-0393397 PASCAL
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TITLE (IN ENGLISH): Role of caveolin in hemodynamic force-mediated **endothelial** changes
AUTHOR: FUJIOKA K.; AZUMA N.; KITO H.; GAHTAN V.; ESATO K.; SUMPIO B. E.
CORPORATE SOURCE: First Department of Surgery, Yamaguchi University School of Medicine, Ube, Yamaguchi, 755-8505, Japan; Anc Department of Surgery, Yale University School of Medicine, New Haven, Connecticut 06510, United States
SOURCE: The Journal of surgical research, (2000), 92(1), 7-10, 32 refs.
ISSN: 0022-4804 CODEN: JSGRA2
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
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AVAILABILITY: INIST-9554, 354000090168670020
AN 2000-0393397 PASCAL
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AB Background. Caveolin has been shown to play an important role in signal

transduction and **nitric oxide** synthase production.
The purpose of this study was to investigate whether caveolin was tyrosine **phosphorylated** or activated by shear stress or cyclic strain in bovine aortic **endothelial** cells (BAECs). Materials and methods. BAECs were subjected to an average of 10% strain at a rate of 60 cycles/min or a laminar shear stress of 10 dyn/cm.sup.2 for up to 4 h. Immunoblotting with anticaveolin **antibody** was performed to assess activation of caveolin. Coimmunoprecipitation of anticaveolin **antibody** with anti-tyrosine **phosphorylation** **antibody** was performed to detect the tyrosine **phosphorylation** of caveolin. Results. Neither cyclic strain nor shear stress at physiologic levels altered the level of caveolin protein. Tyrosine **phosphorylation** of caveolin could not be observed at any time under either cyclic strain or shear stress condition. Conclusion. Although hemodynamic forces alter **nitric** **oxide** synthase production and activate signal transduction, caveolin levels or activity is not altered in **endothelial** cells exposed to shear stress or cyclic strain.

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=> endothelial and (nitrix oxide) and antibody and (phosphorylation or
phosphorylated)
L56             0 FILE CAPLUS
L57             0 FILE BIOTECHNO
L58             0 FILE COMPENDEX
L59             0 FILE ANABSTR
L60             0 FILE CERAB
L61             0 FILE METADEX
L62             0 FILE USPATFULL

TOTAL FOR ALL FILES
L63             0 ENDOTHELIAL AND (NITRIX OXIDE) AND ANTIBODY AND (PHOSPHORYLATION
                  OR PHOSPHORYLATED)
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=> endothelial and (nitric oxide) and antibody and (phosphorylation or
phosphorylated)
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L65          17 FILE BIOTECHNO
L66           3 FILE COMPENDEX
L67           0 FILE ANABSTR
L68           0 FILE CERAB
L69           0 FILE METADEX
L70        1056 FILE USPATFULL

TOTAL FOR ALL FILES
L71        1119 ENDOTHELIAL AND (NITRIC OXIDE) AND ANTIBODY AND (PHOSPHORYLATION
                OR PHOSPHORYLATED)

=> antibody(8A)(phosphorylation or phosphorylated)
L72        2884 FILE CAPLUS
L73        1567 FILE BIOTECHNO
L74          92 FILE COMPENDEX
L75          17 FILE ANABSTR
L76           0 FILE CERAB
L77           0 FILE METADEX
L78        2607 FILE USPATFULL

TOTAL FOR ALL FILES
L79        7167 ANTIBODY(8A)(PHOSPHORYLATION OR PHOSPHORYLATED)

=> l71 and l79
L80          11 FILE CAPLUS
L81           7 FILE BIOTECHNO
L82           1 FILE COMPENDEX
L83           0 FILE ANABSTR
L84           0 FILE CERAB
L85           0 FILE METADEX
L86         288 FILE USPATFULL

TOTAL FOR ALL FILES
L87         307 L71 AND L79

=> l87 and endothelial
L88          11 FILE CAPLUS
L89           7 FILE BIOTECHNO
L90           1 FILE COMPENDEX
L91           0 FILE ANABSTR
L92           0 FILE CERAB
L93           0 FILE METADEX
L94         288 FILE USPATFULL

TOTAL FOR ALL FILES
L95         307 L87 AND ENDOTHELIAL

=> l95 and serine and theronine
L96           0 FILE CAPLUS
L97           0 FILE BIOTECHNO
L98           0 FILE COMPENDEX
L99           0 FILE ANABSTR
L100          0 FILE CERAB
L101          0 FILE METADEX
L102          0 FILE USPATFULL

TOTAL FOR ALL FILES
L103          0 L95 AND SERINE AND THERONINE

=> l95 and serine and threonine
L104          1 FILE CAPLUS
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L105 0 FILE BIOTECHNO
L106 0 FILE COMPENDEX
L107 0 FILE ANABSTR
L108 0 FILE CERAB
L109 0 FILE METADEX
L110 249 FILE USPATFULL

TOTAL FOR ALL FILES
L111 250 L95 AND SERINE AND THREONINE

=> d l104 ibib abs total

L104 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:787754 CAPLUS
DOCUMENT NUMBER: 138:167211
TITLE: Role of platelet **endothelial** form of **nitric oxide** synthase in collagen-platelet interaction: regulation by **phosphorylation**
AUTHOR(S): Chiang, Thomas M.; Woo-Rasberry, Virginia; Cole, Flecia
CORPORATE SOURCE: Veterans Affairs Medical Center, Memphis, TN, 38104, USA
SOURCE: Biochimica et Biophysica Acta (2002), 1592(2), 169-174
CODEN: BBACAQ; ISSN: 0006-3002
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Different pathways have been reported to be involved in the collagen-platelet interaction. The authors previously reported that platelet **endothelial nitric oxide** synthase (eNOS) and the platelet receptor for type I collagen, p65, were closely associated However, the controlling mechanism underlying the generation of NO by eNOS has not been fully explored. Here, Western blot analyses of time-course samples with anti-phosphotyrosine, and anti-**serine/threonine antibodies** showed a marked increase in **serine/threonine phosphorylation** of eNOS during type I collagen-induced platelet aggregation. Meanwhile, the eNOS activity measured by the conversion of [3H]arginine to [3H]citrulline was significantly decreased. The correlation of type I collagen-induced platelet aggregation and the activity of eNOS in the presence of the **serine/threonine** phosphatase inhibitor, okadiac acid, and the tyrosine phosphatase inhibitor, vanadate, were performed with platelet-rich plasma (PRP). The results showed the decrease in eNOS activity by adding okadiac acid correlated with the inhibitory effect on platelet aggregation in a dose-dependent manner. On the other hand, vanadate significantly inhibited platelet aggregation and also inhibited eNOS activity when the concentration of vanadate was >2 mM. These results suggest that **phosphorylation** of **serine/threonine** and tyrosine residues control the activity of eNOS through different mechanisms to affect collagen-induced platelet aggregation.
REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT